VOLUME LXVI NUMBER 4

THE
BOTANICAL GAZETTE

OCTOBER 1918

A CONTRIBUTION TO THE LIFE HISTORY OF IMPATIENS SULTANI

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(WITH PLATES XIV, XV)

This paper is based upon a study of slides made through a series of years for class use in the Botany Department of Wellesley College. The material was taken from greenhouse plants of the rose or bright pink variety of *Impatiens Sultani* Hook. The bright red and light pink varieties also were growing in the greenhouse, but care was taken to collect material from the rose-flowered plants only. Some of the plants from which the flowers were collected were chance seedlings. No attempt was made to determine whether or not these were a pure strain of the rose-colored form. In BAILEY'S (4) *Standard Cyclopedia of Horticulture* the original form of *I. Sultani* is given as a rich scarlet, shades ranging from pink to almost purple being found on hybrids or sports. If this be true, then all the rose-colored forms used for this study are either hybrids or sports.

According to BAILEY the species *I. Sultani* was originally found in Zanzibar and named by HOOKER in honor of the Sultan of Zanzibar. In ENGLER and PRANTL'S *Die Natürlichen Pflanzenfamilien* (16) it is cited from Sierra Leone, Western Africa. It is stated in GRAY'S *Manual* that the Balsaminaceae often contain two kinds of flowers, the large showy ones which rarely ripen

seeds, and small ones which are cleistogamous. *I. Sultani* is not given by ENGLER and PRANTL in their list of species containing cleistogamous flowers, and I was unable to find any cleistogamous flowers on the many plants of this species which were investigated.

The material fixed ranged from very small buds to young fruits. In preparing the buds for fixing, the smallest ones were put up entire; the sepals and petals were removed from all others; and in the largest buds the pistil and stamens were separated. From the flowers and the fruit only the ovaries were preserved and these were trimmed slightly at the angles to allow more rapid penetration of the fixer, which in all cases was Flemming's chromo-acetic solution. In general, the material was sectioned longitudinally and stained with Flemming's triple stain.

Ovary

The ovary consists of 5 carpels with axial placentation. There are several ovules in each loculus and the age of the ovules in a given loculus advances from base to apex, the youngest being at the base of the ovary. At the time when the microspore mother cells are in prophase of the heterotypic division the ovules appear as slightly curving outgrowths from the placenta, with or without any indication of the inner integument (fig. 1). It is apparent that the ovules of *I. Sultani* occur earlier in relation to the development of the anthers than is the case in many other plants. Miss BLISS (8) reports that in *Viola* the ovule initials cannot be detected when the microspore mother cells are in the prophase of the heterotypic division, and similar observations have been made by many other investigators.

As the inner integument begins to appear, a single hypodermal archesporial cell becomes differentiated at the apex of the nucellus (fig. 2). *I. Sultani* agrees with *I. pallida* (Miss RAITT 37) in having but the one archesporial cell, but differs from that species in having no parietal cell cut off from the archesporial cell. According to COULTER and CHAMBERLAIN (15) there is a general tendency to suppress the parietal tissue among monocotyledons and Archichlamydeae. "The suppression of parietal tissue among Archichlamydeae is most extensively displayed by the Ranunculaceae

and its allies rather than by the more specialized groups." Balsaminaceae, one of the higher groups of the Archichlamydeae, shows complete suppression of parietal tissue in *I. Sultani;* in *I. pallida*, however, Miss RAITT (37) describes a parietal cell, but her illustrations are not very conclusive.

From the first the megaspore mother cell is the only hypodermal cell at the apex of the ovule. It is surrounded by the epidermis of the nucellus at its sides and apex, and is bounded by several nucellar cells at its base (fig. 2). The cell is slightly longer than wide and extends almost to the plane of insertion of the inner integument. It keeps pace with the growth of the ovule and continues to occupy all of the nucellus within the epidermis except in the chalazal region. As growth continues it changes from a broad cell to a long narrow one (figs. 2–4, 6).

The nucleus of the megaspore mother cell contains one nucleolus which at first is separated from the chromatic network by a clear area (fig. 2). As division is initiated, the chromatic reticulum forms a more or less complete spirem, separates from the nuclear membrane, collects about the nucleolus, and enters the synaptic stage (figs. 3, 4). On recovery from synapsis the spirem spreads out into the nuclear cavity and very soon exhibits the second contraction stage (fig. 5) similar to that which has been described for *Lilium* and several other angiosperms by ALLEN (2), MOTTIER (32), and others. According to OVERTON (35), this phenomenon does not occur among the majority of the angiosperms.

During this stage the spirem is thick, and at places uneven and massed. Here and there, in the less condensed areas, light streaks show, suggesting either a longitudinal splitting of a single spirem or an approximation of two. The contracted spirem is in contact with the nuclear membrane at several points, but the greater amount of the chromatic material is near the nucleolus at the center of the nucleus. At this time the nucleus is near the micropylar end of the megaspore mother cell. About midway between it and the chalazal end are numerous fibers with blue staining dots scattered among them. This characteristic has been observed in several megaspore mother cells, but its significance was not determined. These fibers played no apparent rôle in the formation

of the spindle or in the development of the cell wall, since they disappear before the first division. However, they may represent an early assembling of the kinoplasmic substance preparatory to spindle formation.

Fig. 6 shows the spirem partially segmented with the nucleolus near the center of the cavity and the segments projecting in from the nuclear membrane. Later the chromosomes become very short and group themselves about the nucleolus (fig. 7). At this time they suggest the tetrads described for many animals and closely resemble those of *Arisaema triphyllum* as figured by ATKINSON (3). A typical bipolar spindle is formed and the bivalent chromosomes when arranged at the equatorial plate appear as very short X's, V's, and Y's.

Two cells separated by a distinct cell wall result from the heterotypic division and form the axial row (fig. 8). The micropylar cell of this row is smaller than the other, and disintegrates very quickly. The chalazal cell grows and is the mother of the embryo sac. An axial row of two cells is not common among the Archichlamydeae. TREUB (43) describes an axial row of two cells for *Viscum articulatum*, and several cases have been reported among the monocotyledons. Miss RAITT (37) states that 4 megaspores are formed in *I. pallida*, but makes no sketch showing them. In her fig. 1, *J*, she shows an ovule containing a large cell which she names the functional megaspore. Between it and the epidermis a small disintegrating cell appears. The sketch closely resembles the appearance of the ovules of *I. Sultani* with an axial row of 2 cells and throws doubt upon her assertion that there is an axial row of 4 cells.

The micropylar cell of the axial row is never large, and is so short-lived that it is easily overlooked, and the embryo sac seems to arise directly from the megaspore mother cell, as in *Lilium*. As the micropylar cell disintegrates and the epidermal cells of the nucellus grow, there appears simply a small blue staining cavity between the embryo sac mother cell and the apical region of the epidermis, as cited by COULTER and CHAMBERLAIN (15) for *Clematis*, and *Helleborus* (GUIGNARD 20), and *Delphinium* (MOTTIER 31). The chalazal cell grows and its nucleus divides, completing the

reduction division and giving rise to the 2-nucleate embryo sac (fig. 9). At this stage there is still something of the disintegrating micropylar cell to be seen, but at a slightly later stage it has entirely disappeared (fig. 10). The embryo sac is thus derived from two megaspores as in *Viscum articulatum* (TREUB **43**) of the Archichlamydeae and in *Trillium* (HEATLEY **26**) and several other monocotyledons.

The two megaspore nuclei move to the opposite poles of the sac and divide (fig. 10). At this stage the sac is vacuolate and continues so until a late 4-nucleate stage (figs. 11–13). The 8-nucleate stage follows rapidly upon the four. Two-, 4-, and 8-nucleate stages have all been found in the same ovary, the 2-nucleate stage being at the base of the loculus. It is very easy, in serial sections, to confuse an early 8-nucleate stage with a late 4-nucleate stage, as the sacs have the same shape and cytoplasmic appearance.

In *Eriocaulon septangulare* (SMITH **40**) the central vacuole first appears at the 4-nucleate stage. In *I. Sultani* the late 2-nucleate sac is vacuolate with large vacuoles between the two nuclei, but there is not one large central vacuole until the female gametophyte has been organized (figs. 10–14, 17). In an early 4-nucleate stage there are several large vacuoles extending along either side of the row of nuclei (fig. 11). During the 4-nucleate stage the sac enlarges, the cytoplasm becomes more dense, and the large vacuoles decrease (figs. 12, 13). By the time the 8 nuclei are formed the cytoplasm is very dense and contains a large amount of stored food, and the vacuoles have become small and inconspicuous (fig. 14). Very soon, however, a large central vacuole appears (fig. 17). The time of the inception of this central vacuole varies considerably. It may arise while the antipodal polar is at the base of the sac, or it may not appear until the polars are in contact and near the egg (figs. 14, 17, 18).

After the organization of the 8 nuclei the egg apparatus soon forms. The egg is more or less pear-shaped, with the larger end extending down below the synergids. The nucleus and greater part of the cytoplasm are in this region and the narrowed part extends up back of the synergids and is vacuolate. It evidently

resembles the egg of *Aster novae-angliae* (CHAMBERLAIN **12**) and that of many other plants as to shape and relation of nucleus and cytoplasm (fig. 17).

The nuclei of the synergids do not always have the same position (figs. 15–17, 19). In the youngest sac of the series (fig. 15) there is a large vacuole at the base of either synergid, with the nucleus above and near the micropylar end. Fig. 16 illustrates a condition in which the synergid nuclei have moved down halfway, and in one cell there is a vacuole on either side of the nucleus, while in the other there is a large vacuole below it but only a small one above it. In fig. 17 one nucleus has moved entirely below the vacuole and is near the membrane at the end of the cell, whereas the other nucleus is still between two or more vacuoles. In a much older embryo sac (fig. 19) the synergids are longer, the nucleus of each is at its base, and a large vacuole appears above the nucleus. At all stages in the growth of the egg apparatus the synergids contain a fairly dense cytoplasm at the apex. It seems clear that the position of the synergid nucleus varies in relation to the age of the sac; that at first it is near the micropylar end of the synergid and above the vacuole; that later it passes the large vacuole and moves down to the opposite end of the cell. When the egg apparatus is mature the two nuclei are at the base of the cells, near the egg nucleus, and just below the large vacuoles (fig. 19).

According to the literature on the subject the position of the synergid nuclei in different plants may vary in relation to the large vacuole of the cell, but I have found no suggestion that the variation in a given species represents different stages in development. CHAMBERLAIN (**12**) gives the situation of the nuclei in *Aster novae-angliae* as varying in position from one end of the cell to the other but most frequently near the middle, the large vacuole being usually at the chalazal end of the synergid. GUIGNARD (**19**) says that in the Leguminosae the vacuole is usually at the base of the cell and the nucleus is central, but the vacuole may sometimes be above the nucleus. BARNES (**6**) in *Campanula americana*, GUIGNARD (**24**) in *Hibiscus Trionum*, and STRASBURGER (**42**) in *Wikstroemia indica von Buitenzorg* find the nucleus above the

vacuole; while PACE (**36**) finds the synergid vacuoles of *Parnassia* in various positions.

The antipodals are surrounded by denser cytoplasm than is present above them, and they appear rarely as separate cells with delicate walls separating them (fig. 18), or, as is more commonly found, the mass of cytoplasm with the 3 nuclei is more or less cut off from the rest of the sac by a membrane but the cells are not separated. In either case they are but short-lived and disappear soon after the egg apparatus is formed. The embryo sac then persists for a long time with but 5 nuclei. Miss RAITT says that the antipodals in *I. pallida* cannot be distinguished with certainty and are evidently transitory. This ephemeral nature of the antipodals is common among many of the angiosperms. In *Striga lutea* (MICHELL **29**) the 3 antipodal cells begin to disintegrate before fertilization. In *Richardia africana* (MICHELL **30**) the disintegration is somewhat earlier, evidently more nearly like *I. Sultani*. In this species the antipodals were never found to increase in size or number and grow into the adjoining tissue, as has been described by CHAMBERLAIN (**12**) and OPPERMAN (**34**) for *Aster novae-angliae* and by others for various plants.

Very quickly after the 8 nuclei of the sac have been formed the antipodal polar moves up toward the micropylar polar and the 2 nuclei remain near each other at a short distance below the egg nucleus for some time. The nuclei may or may not be spherical, but they always contain a prominent nucleolus with a small highly refractive spot at the center. The chromatic substance of the polar nuclei is small in amount and forms either a delicate network lying just within the nuclear membrane, or a few strands radiating out from the nucleolus. Most of the food stored in the sac at an earlier period disappears before the female gametophyte reaches maturity and the sac becomes very vacuolate, with only a layer of cytoplasm at its periphery and surrounding the nuclei in the micropylar half of the sac (fig. 37).

During the later development of the embryo sac its shape becomes much changed (figs. 17, 19, 37). While the antipodals are still present the sac is a little over three times longer than wide, with the micropylar and antipodal ends both rounded in outline

(fig. 17). After the antipodal cells disintegrate the basal region of the sac grows down into the chalaza. The growing portion is blunt or more or less triangular in outline, and but little narrower than the sac just above. The antipodal growth continues until the sac is over five times as long as it is wide (fig. 19). Following the development of this antipodal haustorium the sac widens in the region of the polar nuclei and assumes its mature shape (fig. 37).

During the origin and development of the female gametophyte the megasporangium has been undergoing marked changes. The ovule begins to curve before the megaspore mother cell appears, and by the time its nucleus has reached the segmented spirem stage the ovule has attained the anatropous position. At this time the inner integument extends beyond the nucellus and forms a fairly deep micropyle (figs. 1–4, 6). The outer integument arises from the lower two-thirds of the inner integument, appearing as a swelling from the outer part of the latter (fig. 6). This swelling increases greatly in breadth and grows up until its apex is on a line with the tip of the inner integument. It never grows beyond this point to aid in forming the micropyle, and the two integuments become distinct only at the summit (figs. 9, 37).

The origin of the outer integument in *I. Sultani* differs from the majority of plants and from the other species of *Impatiens* that have been studied. Miss RAITT (**37**) in *I. pallida* and GUIGNARD (**22**) in *I. parviflora* both show the outer integument arising from the basal portion of the ovule and remaining throughout its length distinct from the inner. As a result of his study of *I. balsamina*, BRANDZA (**9**) states that the Balsaminaceae have but one integument, but BRUNOTTE (**10**) disagrees with BRANDZA and gives two integuments for this family. According to LONGO,[1] as cited by Miss RAITT, this is the rule for the genus *Impatiens*, but the origin of the outer integument and its extension at the micropylar region appear to vary. From my study of the ovule of *I. Sultani* it can readily be seen how BRANDZA thought there was but one integument if *I. balsamina* is similar to *I. Sultani* in having the outer integument an outgrowth of the inner integument with only their tips free.

[1] LONGO, B., Richerche su le *Impatiens*. Annali Bot. **8**:65–77. 1909.

In *De l'ovule*, WARMING (45) notes a few exceptions to the usual order of development of the integuments and gives *Viola*, *Ficus*, *Convallaria*, and *Orchis* as having two integuments which appear to grow as a single organ, and *Tropaeolum* as having at first two integuments which later appear as one. WARMING quotes STRASBURGER as saying that in *Delphinium* the integuments originate as one and elevate themselves as a unit; later at the summit the two integuments become distinct. Judging from his figure the conditions in *Delphinium* are much the same as in *I. Sultani*.

As given earlier, the megaspore mother cell when it arises is completely surrounded, except at its base, by the epidermis of the nucellus, and the developing embryo sac also continues to lie in direct contact with the epidermis (figs. 2–4, 6, 8, 9). During the 2-nucleate stage of the embryo sac the epidermis begins to break down. The disintegration first appears as a flattening of the cells and nuclei just below the apex, and then extends gradually to the base of the embryo sac (figs. 9, 10, 14, 37). In *Oxalis corniculata*, according to HAMMOND (25), the epidermis, which in this case serves as a tapetum, begins to disintegrate before the 2-nucleate embryo sac is formed.

The apical cells of the epidermis are often longer-lived than those just below them, for it is quite common to find two or three cells surmounting the embryo sac and connected by only a line with those still persisting about the center of the sac (fig. 12). A somewhat similar appearance has been described by SMITH (40) for *Eriocaulon septangulare*, where "the nucellar tissue lateral to the megaspores breaks down and is absorbed by the growing embryo sac. A few of the apical cells of the nucellus persist for a long time and enlarging assume the appearance of a tapetum. These too are ultimately absorbed and the embryo sac abuts directly upon the inner integument and micropyle." In *I. Sultani*, however, these apical epidermal cells do not enlarge and seem to have no special function.

While the epidermis continues to disappear it leaves but a line around the upper half of the sac (fig. 10). As the disintegration progresses downward, the cells near the base of the sac possess their normal tabular shape, while those nearer the middle of the

sac are narrowed and pointed. The nuclear substance in the pointed cells is dense, the nuclei are often flattened, and the cell content stains but slightly. The pointed end of the layer often becomes free from the embryo sac (fig. 13). As this layer is disappearing it is not always in close contact with the tapetum (fig. 10), and I infer that the disappearance of the epidermis is due, not to its being crushed between the tapetum and the enlarging embryo sac, but rather to the fact that it is being absorbed by the embryo sac. The basal portion of the epidermis continues to exist until after the 8-nucleate sac is formed (fig. 14).

Not only is the entire epidermis absorbed eventually but the nucellus beneath the embryo sac also. During the early stages in the development of the embryo sac the cells of this part of the nucellus are similar to those of the interior of the integuments, but beginning with the 4-nucleate stage, or occasionally earlier, they become stringy in appearance, with their long diameters in line with that of the sac. This strand of cells extends down to the chalaza which is composed of a tissue of regular, compact, densely staining, isodiametric cells (figs. 12, 14, 37). Many of the nuclei of the "stringy" cells show signs of disintegration. They become dense, lose their rounded outline, and appear elongated. These cells are bounded at the sides by the tapetum (fig. 14). The antipodal region of the sac absorbs this tissue and pushes down to the nutritive cells of the chalaza, thus completing the absorption of the entire nucellus (fig. 37). *I. Sultani* agrees with *I. parviflora* (GUIGNARD **21**) and *I. amphorata* (LONGO **28**) in having the embryo sac absorb the nucellus and thus come in contact with the micropyle and the inner integument. These species of *Impatiens*, therefore, agree with the Compositae (GOLDFLUS **17**) in the early disappearance of the nucellus.

As described by GUIGNARD (**22**), RAITT (**37**), LONGO (**28**), and BRUNOTTE (**10**) for *Impatiens*, and by others for various angiosperms, the epidermis of the inner integument forms the tapetum of regular tabular cells, which, in *I. Sultani*, extend from the base of the micropyle to a considerable distance below the base of the developing embryo sac (figs. 9, 10, 14). The tapetum loses its uniform character as early as the 2-nucleate stage of the embryo sac, when a densely staining substance appears between the tapetal

cells near the base of the micropyle and the contents of these cells stain diffusely. This appearance also extends out laterally and at this time up to the tip of the inner integument (fig. 9). The cells are crowded and their cytoplasm and nucleoplasm stain so diffusely that no attempt was made to represent their appearance in a sketch. As will be seen later, these cells break down during endosperm formation, and this doubtless represents an early stage in their disintegration. This characteristic progresses chalazally in the tapetal cells as the embryo sac develops and reaches the basal cells during early endosperm formation.

In an 8-nucleate stage the lower half of the tapetum is still normal and contains a decidedly granular cytoplasm and nucleoplasm which suggests the presence of food particles. This granular appearance is not visible in the cytoplasm of the other cells of the inner integument, although they stain more densely than do those of the outer integument. GUIGNARD (**22**) says that a nitrogenous substance accumulates in the tapetal cells of *I. parviflora* and that in all of the Balsaminaceae there is this proteid layer of tabular cells.

BILLINGS (**7**) believes with most students that the tapetal layer, whether from the inner integument or the nucellus, serves a nutritive function, dissolving and absorbing nutriment from the surrounding integument, and that its function is not simply protective, as given by HEGELMAIER (**27**). VANDENDRIES (**44**) in a study of the Cruciferae finds the tapetum a part of the inner integument, but believes it plays only a protective function. One reason given is that in the antipodal region, where the tapetum is separated from the sac by a small mass of nucellar cells, it presents the characteristic appearance of young and active tissue. This reasoning does not seem conclusive to me, since it may well be that this was a region of considerable food and that here the active cells of the tapetum digested and absorbed it and then passed it up to the embryo sac. In *I. Sultani* the tapetum persists longer at the antipodal end and it is here that growth takes place until the sac reaches the chalaza and passes slightly beyond the end of the tapetum.

BALICKA-IWANOWSKA (**5**) studied certain "Gamopetales" and described the tapetum. In agreement with CHODAT (**13**) and most recent writers, he does not believe that the tapetum is for protection,

as it is wanting in the vicinity of the haustorium, which does not possess cell walls and would therefore appear in need of protection. He thinks that the tapetal cells possess a ferment in their mucilaginous content and exercise a digestive function, for they persist while the neighboring tissue disintegrates, and they surround the parts which are in the process of rapid growth.

Extending through the raphe is a strand of cells which is surrounded, except at the ends, by a layer of cells with cutinized walls. This strand terminates at the chalaza in the tissue of regular compact cells. It is into this that the antipodal region of the sac pushes (fig. 37). In looking at the figure it will be seen that the outer layer of cutinized cells ends just as it passes this area, and on the inner side the layer ends almost in contact with the antipodal end of the tapetum. It thus forms a protective covering to the conducting tissue as it passes to the chalazal haustorium. No true vessels were ever observed in this strand of conductive cells.

As noted earlier, the antipodal nuclei disappear before the haustorium develops, thereby giving rise to the unusual condition of a haustorium unaccompanied by nuclei. In none of the literature studied was I able to find any record of a similar condition. In the cases where a haustorium has developed at the antipodal region before fertilization had occurred, it is usual for the antipodal nuclei to be present in the haustorium formed. An interesting example of this is given by SOUÈGES (41) for the Solanaceae, where a pocket is formed at the basal part of the embryo sac and the antipodals take their place in the bottom of this and their digestive juices diffuse into the tissue beneath and dissolve out a cavity. The process of dissolution of the tissue varies among the different members of the family from a simple disjunction of the digestive layer of cells to a chalazal cavity whose capacity is comparable to that of the embryo sac itself, as in *Lycopersicum esculentum*.

Stamen

The flower possesses 5 stamens whose anthers are connivent and form a hood over the pistil. In a cross-section of a bud the sides of two adjacent anthers show as having their cells in contact, and this region appears as solid tissue. Each anther contains 4 micro-

sporangia. Fig. 20 shows a cross-section of one-quarter of an anther when the nuclei of the microspore mother cells are in the synaptic state. The walls of the cells of the epidermis are cutinized and thicker than those of the other cells. The wall of the microsporangium consists of two distinct regions, the outer irregular portion made up of 1–5 layers of nearly isodiametric cells, and an inner region about 2 cells thick, the cells of which are flattened tangentially. This flattening, doubtless, results from the pressure caused by the growth of the archesporial and tapetal cells.

The microspore mother cells are separated from this inner wall by the tapetal cells. The latter, however, are not limited to the peripheral region, but extend into the mass of sporogenous cells and in some cases ramify entirely through the loculus, occupying more than one-half of the sporangial cavity. The origin of the tapetum was not definitely determined, but it seems highly probable that it arises from the sterilization of sporogenous tissue rather than from the inner cells of the wall, and that all of the sterile cells within the sporangium are of the same ancestry. The tapetal cells vary in size, many of them being as large as the microspore mother cells. They are binucleate and are more vacuolate than are the functional spore mother cells. The two nuclei of a single cell are usually side by side either at the center of the cell or at one end. Each nucleus contains one nucleolus or occasionally more.

CALDWELL (11) describes a condition for *Lemna minor* in some respects similar to that just outlined. He finds that during the early stages of the heterotypic division the cells of the tapetum sometimes divide and form groups of cells which project into the mother cell region; that the number of microspore mother cells is not reduced by the presence of the tapetal cells, although only a comparatively few developed spores, the others disorganizing and aiding the tapetum in nourishing the functional mother cells. In *I. Sultani* only about half the number of microspore mother cells arise that one would expect from the size of the sporangium. As indicated, not all of the tapetal cells arise at the periphery of the sporogenous mass, but many of them originate side by side with the microspore mother cells. The small number of microspore

mother cells may doubtless be related to the probable hybrid nature of the form of *Impatiens Sultani* used. The distinction between the functional and non-functional sporogenous tissue can clearly be seen at an early stage, and still shows distinctly when the spore mother cells are in synapsis. The latter are still angular and are only just beginning to separate (fig. 20).

As is customary at the time of synapsis, the chromatic substance is massed against the nuclear membrane, with the single large vacuolate nucleolus projecting out from one side. As the prophase of the heterotypic division advances and a delicate spirem fills up the nuclear cavity, the cells become almost entirely free and round off, while the tapetal cells still remain somewhat angular in outline and often contain more than the 2 nuclei of the earlier stage (fig. 21). Their nuclei have become granular, lack a nucleolus, and stain more densely than before. In general, the entire mass of cells has separated from the wall. The ovule at this age shows no sign of the inner integument.

As the anther increases in size, the microspore mother cells become entirely rounded off and the spirem takes on a double appearance, whether due to a splitting of a single spirem or an approximation of two was not apparent. At this time the spirem is thicker and less delicate than the spirem immediately following synapsis (figs. 21, 22). The cytoplasm has an obscurely radiate appearance, being densely granular about the nuclear membrane and more vacuolate toward the cell membrane. The spirem thickens, becomes irregular, and segments transversely into bivalent chromosomes. The majority of the segments come to lie against the nuclear membrane and show clearly their double nature in the forms of X's, Y's, and V's. They are rough in outline and are connected here and there by delicate threads (fig. 23), similar to those figured by MOTTIER (33) for *Acer Negundo* and *Staphylea trifolia*. The nucleolus still persists and at this stage there may be two, a large and a small one.

The granular area surrounding the nucleus has become still more marked and closely resembles the kinoplasmic region described by ALLEN (1) for the pollen mother cells of *Larix* and by numerous other writers. The peripheral cytoplasm with its large meshes

draws away from the cell membrane at various points. At this stage cellulose begins to be deposited about the cell and when the chromosomes have reached the equatorial plate a broad cell wall of cellulose is formed (fig. 27). As the chromosomes shorten and thicken, they become smooth in outline and numerous fibers heavier and longer than those mentioned for an earlier stage (fig. 23) appear within the nuclear cavity. These fibers seem to have no apparent relation to the fibers of the kinoplasmic region, although at this time the nuclear membrane has become indistinguishable from the cytoplasm at various places (figs. 24, 25). The fibers are tufted and may extend from a given chromosome across the nuclear cavity to other chromosomes or to the nuclear membrane. At this time no nucleoli are visible within the nuclei and there is the possibility that their substance has assisted in the formation of the fibers.

After the nuclear membrane has entirely disappeared many fibers appear about the chromosomes (fig. 26). They extend beyond what was the original area of the nucleus and doubtless there has been a union of intra- and extra-nuclear fibers in the formation of the multipolar spindle (fig. 26). By the time metaphase is reached the spindle has become sharply bipolar and extends across the entire cell (fig. 27). The cytoplasm surrounding the spindle has lost the dense granular appearance of the early prophase stages and stains less densely than does the peripheral region.

While I am not willing to make an unqualified statement regarding the number of bivalent chromosomes, it seems most probable from the study of the heterotypic divisions in the megaspore and microspore mother cells that the haploid number is 7, as I have been unable to count more than that number. No stages in microsporogenesis were obtained between metaphase of the heterotypic division and the tetrads following the homotypic division.

When the tetrads are formed the microspores are surrounded by the very thick cellulose wall of the mother cell. Each microspore is a little over 3 times longer than wide and possesses a reticulated membrane. At this time its nucleus is not spherical,

but simulates the outline of the cell and its chromatic material is distributed unevenly throughout the nuclear cavity. Several large masses of chromatin are mingled with chromatic threads which extend out from them in various directions (figs. 28, 29).

The loculi containing these tetrads also contain densely staining tapetal cells. These cells still have large vacuoles, but the cytoplasm stains more densely than in earlier stages and their nuclei contain one nucleolus each. Outside the tapetum is the flattened layer of cells, and beyond this the remainder of the wall is still undifferentiated.

Later, when the microspores have broken away from the old mother wall, the endothecium with its spirally thickened cell walls forms just beyond the flattened layer. The tapetal cells, in general, are still in good condition, some of them centrally located having increased very greatly in size and number of nuclei. Compare the tapetal cell (fig. 33) which was magnified 810 times with the microspore (fig. 30) which was taken from a loculus of the same age and magnified 1620 times. The number of nuclei in these tapetal cells may reach as high as 11 or more. These unusually large cells show stages in disintegration, and it is difficult to find one which has not begun to break down. A large number of nuclei, ranging from 6–13 in the tapetal cells of *Hepatica acutiloba*, has been reported by COULTER (**14**). SCHAFFNER (**39**) in his description of *Typha latifolia* states that the tapetal cells increase greatly in size while the tetrads are forming, but speaks of but 2 nuclei being formed in each cell.

When the microspores escape from the tetrad the chromatin of their nuclei consists of heavy, anastomosing strands which soon give rise to several distinct masses of chromatin connected with each other by more or less delicate threads. In by far the greater number of cases, if not always, these masses correspond in number with the haploid number of chromosomes (fig. 30). No nucleolus is visible at this time. There is evidently no true spirem formed in the division of the nucleus to form the generative and tube cells, but the rather imperfect reticulum of the very young microspores gives rise directly to the chromosomes at a considerable time before the organization of the spindle. It is a very common occurrence

to find all the microspores of an anther in an apparently resting stage and showing distinctly 7 chromatic masses.

The microspore divides and forms a more or less vacuolate 2-celled pollen grain (figs. 31, 32). The tube nucleus is normally spherical and lies more or less near the center of the developing pollen grain (figs. 32, 34, 35). The generative cell occupies various positions within the cytoplasm of the tube cell, and at all times its nucleus is smaller and its reticulum is much less delicate than that of the tube nucleus. The pollen grain grows and its cytoplasm becomes densely filled with food granules. At this time the generative cell may either be attached to the wall of the pollen grain or lie free in the cytoplasm (figs. 34, 35).

When the anther is ready to dehisce, it is impossible to distinguish the cytoplasm of the generative cell, and its nucleus has changed from nearly spherical to a very slender lunate form (fig. 36). At first it was thought to be a sperm nucleus, but while these crescent-shaped nuclei are very characteristic of the developing male gametophyte, no more than one to each pollen grain was ever found. When the nucleus is in this condition it contains several chromatic masses in the center and stains a diffuse yellow at either end. In all cases where the nucleus could be seen clearly throughout its entire length 7 chromatic masses were visible (fig. 36). The mature pollen grains, in longitudinal section, present an almost rectangular appearance and possess 4 germ pores, one at each corner. No instances of the division of the generative cell while still within the anther were discovered, and it is doubtful whether this division takes place until some time after pollination.

Ovule after pollination

The pollen tube enters the embryo sac after the chalazal haustorium has developed. Many of the embryo sacs of a given ovary were found containing the pollen tube contents in their micropylar region. While no experiments were made to eliminate all chance of cross-pollination, the conditions under which these plants were grown in the greenhouse render it very probable that many of the flowers were self-pollinated. The pollen tube enters the embryo sac at one side of the filiform apparatus and either

continues down the same side of the sac near to the region of the egg nucleus or crosses over the synergid and extends down the other side (figs. 37, 38). After this has occurred it is difficult to find both of the synergid nuclei. Doubtless one of them soon becomes disorganized, due to the effect of the presence of the pollen tube. SMITH (40) describes the pollen tube of *Eriocaulon* as either passing through a synergid or between the two without destroying them.

In *I. Sultani* the tube nucleus was usually visible in the embryo sac, but it was often difficult to discover the sperms, due to their small size and also to the presence of many small densely staining bodies which often suggested parts of nuclei but were possibly food particles. The sperms are coiled or spiral in outline as they approach the egg and polar nuclei. In fig. 38 the two sperms are both near the egg nucleus, one is directly over the latter and the other is at its side, still in the dense strand of cytoplasm which marks the path of the pollen tube contents, and doubtless is on its way to the two polar nuclei. No sperm cytoplasm is visible and the nucleus is a spiral body made up of dark and light areas, the former of which are doubtless masses of chromatin. The character of the sperm shown in fig. 39 differs somewhat from those in the preceding figure. Here a sperm nucleus is situated one at either side of the egg nucleus; the one at the right is coiled tightly and shows no distinction between chromatic and clear areas, but stains a clear light blue. It was doubtless on its way through the cytoplasm at the side of the egg to the endosperm nucleus lying directly below the egg.

No stages in the actual fusion of the egg and sperm were seen. The fertilized egg differs so slightly from the unfertilized one that it is difficult to decide in a given case whether or not fertilization has occurred. In general, however, the fertilized egg increases slightly in size and its limiting membrane is more conspicuous than it is in earlier stages (fig. 40). Figs. 38 and 40 have the same magnification and the increase in size is evident.

It seems probable that the polar nuclei unite at an early stage in the fusion of the sexual nuclei. The nuclear membranes of the two polars break down where they come in contact and one of the nucleoli passes over into the other nucleus (figs. 41, 42). Both

nucleoli possess one or more vacuoles. What appears to be a later stage in the fusion of the two polars is given in fig. 43. A dense granular mass, the entering nucleolus, seems to be in vital contact with the nucleolus of the receiving nucleus and gives the impression of giving of its substance to help in the formation of the nucleolus of the resulting endosperm nucleus. The characteristically large vacuole of the primary endosperm nucleolus has already appeared. Not all of the entering nucleolus fuses with the receiving nucleolus, however, for coarse strands radiate out from the dense mass of nucleolar substance and appear to be adding to the reticulum of the nucleoplasm. A similar radiating mass has been observed in one of two polars. In this case it might represent a stage in the fusion of the second sperm with one of the polars before the two polar nuclei had united. Similar masses have also been seen in the megaspore mother cell, and here also the nucleolus doubtless contributes to the chromatic substance.

The fusion of the two polars has been figured by numerous writers for many different plants. In *Nicotiana Tabacum* (GUIGNARD **23**) the two nucleoli remain distinct in the fusion nucleus for some time before fusing. VANDENDRIES (**44**) figures for *Cardamine pratensis* two nucleoli within the primary endosperm nucleus with a sperm against one side of it. He says that when the pollen tube enters the cavity of the embryo sac the two polar nuclei have begun to fuse but the nucleoli are still distinct. In *I. Sultani*, however, the fusion of the two polar nuclei begins relatively much later and takes place very quickly. In describing the fusion of the two polars of *Arisaema triphyllum*, GOW (**18**) says that the fusion endosperm nucleus frequently contains two nucleoli.

It seems highly probable that after the primary endosperm nucleus is formed the second sperm unites with it. By this time the sperm nucleus appears to be larger than it was in earlier stages. In fig. 44 it is just at the point of piercing the nuclear membrane, and in fig. 45 it is within the primary endosperm nucleus and lying either above its nucleolus or within it. It was impossible to determine if the second sperm, in all cases, waited until the primary endosperm nucleus was formed before becoming functional. GUIGNARD (**23**) is convinced that in the Malvaceae the time of

formation of the secondary nucleus is constant for a given genus. In *Lavatera* and others it is formed before fertilization, while it is formed after in *Hibiscus*.

Following fertilization a micropylar haustorium is formed. The primary endosperm nucleus divides before the division of the fertilized egg. After a few divisions have taken place several of the resulting endosperm nuclei pass up through the micropylar part of the embryo sac (figs. 46, 47) out into the micropyle and form a haustorium which emerges from the micropyle and crosses over the space between the latter and the funiculus (figs. 49*a*, *b*). As the haustorium encounters the funiculus it either extends along the funiculus some little distance before entering it or penetrates it immediately and branches freely within its tissue (fig. 53).

In the early stages, as the endosperm nuclei pass through the upper part of the sac, they are surrounded by cytoplasm rendered dense by the presence of a large amount of food substance and consequently the nuclear membrane is entirely obscured (figs. 46, 47). The densely staining filiform apparatus, all that remains of the two synergids, is still present, but it is more widely separated from the enlarged part of the embryo sac than in earlier stages (fig. 47). The narrow micropylar part of the sac where the synergids of the mature embryo sac were situated has widened and encroached upon the small disorganizing cells of the inner integument. These cells have now disappeared except for a few remains and large regular cells limit the micropylar cavity into which the sac has pushed (cf. figs. 9, 14, 47). At this stage the fertilized egg is still undivided and several endosperm nuclei lie below as well as above it. These nuclei are not shown in fig. 47, as they occurred in a different section from the one sketched.

In the later stages in the development of the micropylar haustorium but few nuclei are present in it. These are very large and contain a large nucleolus and stain bright red with safranin (fig. 53). This haustorium was seen to persist up through the oldest stages studied, namely, those containing embryos with radicle and cotyledons differentiated.

The micropylar haustorium of *I. Sultani* differs from that of *I. amphorata*, as described by LONGO (**28**), in not entering the

outer integument. It simply pierces the funiculus and branches extensively within it.

As in *I. amphorata* (LONGO 28), *I. Sultani* possesses a chalazal haustorium as well as a micropylar one. This haustorium is much less extensive than is the micropylar one. One of the endosperm nuclei at the antipodal region becomes very large, and with its surrounding cytoplasm forms a long cell which pierces through the sac and one end of it enters the chalazal tissue, while the other end remains in contact with numerous normal endosperm cells (figs. 50, 51, 53).

It was difficult to secure a vertical section through this haustorium, as a series of sections which cut through the embryo vertically would section the haustorium somewhat diagonally. Fig. 50 shows the outline of the cell, but no nucleus, while fig. 51 shows the embryo sac portion with a large nucleus, but the chalazal part was cut so that all connection between chalazal tissue and haustorium was lost. This large haustorial cell contains a densely granular cytoplasm and is doubtless active in conveying nutriment from the chalaza to the endosperm. This haustorium does not remain active as long as the micropylar one. By the time the cotyledons of the embryo have become differentiated it is not so prominent as in earlier stages, while the micropylar haustorium still seems very active (fig. 53).

The endosperm develops more rapidly at the micropylar and chalazal regions than at the sides of the sac. A layer of cytoplasm with a single row of free nuclei persists at the sides for some time, while at the poles of the sac walls come in early to separate the nuclei (figs. 49*a*, 50, 51). With the presence of two rows of nuclei at the sides, walls form about the outer layer of nuclei, while those of the inner layer remain free (fig. 48) until some time later, when they are separated by walls, and the endosperm is composed entirely of cells (fig. 53). The size and shape of the embryo sac have undergone marked changes during endosperm formation. At the time of fertilization the outline of the sac is similar to that shown in fig. 54, with the early antipodal haustorium opposite the micropylar part of the sac. Later, during early endosperm formation, the sac elongates and widens slightly at the micropylar region. Below

this it enlarges considerably on the side away from the raphe, and the sac becomes asymmetrical (fig. 55). This lateral growth continues until it extends even beyond the original position of the antipodal haustorium, with the result that the chalazal haustorium of endosperm origin comes to lie at the side of the sac rather than at its base, and the embryo sac extends below the chalaza (fig. 53).

The embryo develops less quickly than does the endosperm. As stated earlier, the fertilized egg does not divide until after many free endosperm nuclei have been formed. The proembryo becomes differentiated into suspensor and embryo early in its development. By the time the embryo consists of several cells the suspensor, in a longitudinal section, shows but two cells. The cell adjoining the embryo is broader than long and the terminal one is but slightly longer than wide (fig. 49*a*). The suspensor, doubtless on account of the very effective micropylar haustorium, appears to be but a short-lived organ. It neither elongates nor becomes bladder-like, as is the case for many of the angiosperms, but soon breaks down and disappears. It shows signs of disintegration before the cotyledons of the embryo appear (fig. 52), and by the time they are differentiated the suspensor has disappeared. The embryo develops at the tip of the suspensor and by the time the endosperm consists of two layers of cells about the periphery of the embryo sac the radicle and two cotyledons have become differentiated (figs. 53, 56).

The embryo and the chalazal haustorium do not occur in the same vertical plane, therefore it is impossible to secure satisfactory sections of the two from the same embryo sac. In the oldest stage studied a band of endosperm cells lines the sac (figs. 53, 56). The endosperm lies in close contact with the axis of the embryo and the sides of the cotyledons, but it is separated from the chalazal end of the embryo by a large cavity. Unfortunately, through lack of study of the seeds of *I. Sultani*, I was unable to determine the fate of the endosperm. According to GUIGNARD (**22**), a thin layer of endosperm remains undigested in the mature seed. BRUNOTTE (**10**), from his study of the Balsaminaceae, believes that the descriptions of the systematists for the mature seed should be changed.

Instead of describing the seed as having no endosperm, he would say that there is a small amount of endosperm present.

Summary

1. The ovule possesses but one hypodermal archesporial cell.
2. The archesporial cell is also the megaspore mother cell.
3. An axial row of two cells is formed. The chalazal cell is the mother of the embryo sac.
4. A normal 8-nucleate sac is formed.
5. There is a variation in the position of the synergid nuclei, due to their age.
6. The two polar nuclei come in contact directly beneath the egg and do not fuse until after the pollen tube has entered the embryo sac.
7. The 3 antipodals may be either 3 cells or a group of 3 nuclei cut off from the upper region of the sac by a membrane.
8. The antipodals disappear soon after the egg apparatus is formed.
9. The megaspore mother cell and the early 2-nucleate embryo sac are bounded at the apex and sides by the nucellar epidermis.
10. The embryo sac absorbs the nucellar epidermis and by means of an antipodal haustorium absorbs all of the nucellus between the sac and the chalaza.
11. The tapetum is derived from the inner layer of the inner integument.
12. The outer integument arises from the inner integument.
13. Binucleate tapetal cells surround the microspore mother cells. They also extend into the mass of sporogenous cells and separate the functional mother cells into groups.
14. The nucleus of the generative cell of the pollen grain apparently does not divide before pollination.
15. The pollen tube enters the embryo sac along the side of the filiform apparatus and extends down one side of the embryo sac until it is near the egg nucleus.
16. Two coiled sperm nuclei are often seen near the egg nucleus.
17. It seems very probable that triple fusion occurs.

18. An extensive micropylar haustorium and a more simple chalazal one develop from the endosperm.

19. The embryo possesses a short suspensor.

20. The bivalent chromosomes in both megasporogenesis and microsporogenesis show X's, Y's, and V's.

21. A multipolar spindle appears in the prophase of the heterotypic division in microsporogenesis.

22. The nucleus of the microspore has but a short period of rest; the prophase of division is initiated early and persists for some time.

In conclusion, I wish to thank Professor MARGARET C. FERGUSON for her careful reading of this paper and for her very helpful suggestions.

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EXPLANATION OF PLATES XIV, XV

All figures were drawn with the aid of an Abbé camera lucida and are reduced one-half in reproduction. The number accompanying the description of each figure indicates the magnification before the reduction. The lettering of the figures is as follows: *ii*, inner integument; *oi*, outer integument; *mc*, megaspore mother cell; *n*, nucleolus; *f*, funiculus; *ma*, micropylar cell of axial row; *e*, epidermis of nucellus; *t*, tapetum; *s*, stringy cells of the nucellus; *en*, egg nucleus; *sn*, synergid nucleus; *cv*, central vacuole; *p*, polar nucleus; *mm*, microspore mother cell; *dn*, disintegrating nuclei; *tn*, tube nucleus; *gn*, generative nucleus; s^1, sperm nucleus; s^2, sperm nucleus; *fa*, filiform apparatus; *ptc*, pollen tube contents; *rp*, receiving polar; *m*, micropyle; *ah*, antipodal haustorium; *mh*, micropylar haustorium; *ch*, chalazal haustorium; *sp*, suspensor; *c*, chalaza; *ed*, endosperm; *vs*, vascular strand; *r*, radicle; *co*, cotyledon; *w*, wall; *pe*, primary endosperm nucleus; *ra*, raphe; *cu*, cells with cutinized walls; *nrp*, nucleolus of receiving polar; *edn*, endosperm nuclei.

PLATE XIV

Fig. 1.—Vertical section of ovule before integuments have appeared; ×828.

Fig. 2.—Vertical section of ovule showing origin of inner integument and large megaspore mother cell; ×810.

Fig. 3.—Vertical section of ovule slightly older than fig. 2; ×810.

Fig. 4.—Vertical section of ovule with nucleus of megaspore mother cell in synapsis; ×810.

Fig. 5.—Vertical section of megaspore mother cell with nucleus in second contraction stage of prophase of heterotypic division; ×1620.

Fig. 6.—Vertical section of ovule showing origin of outer integument and segmented spirem of megaspore mother cell; ×500.

Fig. 7.—Vertical section of megaspore mother cell showing tetrad formation in nucleus; ×1300.

Fig. 8.—Vertical section of nucellus after 2-celled axial row has been formed; ×1300.

Fig. 9.—Vertical section of portion of ovule containing 2-nucleate embryo sac; ×1000.

Fig. 10.—Vertical section of 2-nucleate embryo sac with surrounding nucellus and tapetum; nuclei of embryo sac in prophase of division; ×810.

Fig. 11.—Vertical section of young 4-nucleate embryo sac; combination of 3 serial sections; ×810.

Fig. 12.—Vertical section of slightly older sac with nucellus and disintegrating epidermis; ×810.

Fig. 13.—Vertical section of still older 4-nucleate embryo sac with basal portion of epidermis still intact; ×1000.

Fig. 14.—Vertical section of early 8-nucleate embryo sac with nucellus at base and tapetum at either side; ×1000.

Fig. 15.—Vertical section of early stage in formation of egg apparatus; synergid nuclei each above a large vacuole; ×1000.

Fig. 16.—Vertical section of micropylar region of embryo sac showing 2 synergids; synergid nuclei have reached center of the 2 synergid cells; ×1000.

Fig. 17.—Vertical section of 8-nucleate embryo sac before the 2 polar nuclei have reached their position directly beneath egg; combination of 2 serial sections; ×1000.

Fig. 18.—Vertical section of antipodal region of 8-nucleate sac before antipodal polar has moved toward micropylar polar; the 3 antipodals show as distinct cells; combination of 2 serial sections; ×1620.

Fig. 19.—Vertical section of embryo sac after antipodals have disappeared and antipodal haustorium has begun to develop; micropylar tip of sac was covered by inner integument; ×1000.

Fig. 20.—Cross-section of microsporangium while spore mother cells are in synaptic stage; ×500.

FIG. 21.—Cross-section of contents of microsporangium soon after chromatic substance of microspore mother cells has formed a spirem following synapsis; ×500.

FIG. 22.—Section of microspore mother cell slightly older than those in preceding figure; nucleus contains spirem showing its double nature; ×1620.

FIG. 23.—Section of microspore mother cell soon after spirem has segmented; 2 nucleoli present, small one directly over large one was omitted from sketch; ×1620.

FIGS. 24, 25.—Sections of 2 microspore mother cells after bivalent chromosomes have shortened and become smooth in outline and intranuclear fibers have appeared; in fig. 25 a thick wall is beginning to form about the mother cell; ×1620.

FIG. 26.—Section of microspore mother cell with multipolar polyarch spindle; heterotypic division; ×1750.

FIG. 27.—Section of microspore mother cell showing bipolar spindle of heterotypic division; bivalent chromosomes arranged at equatorial plate; ×1620.

FIG. 28.—Cross-section of tetrad; ×1620.

FIG. 29.—Vertical section of tetrad with but 2 of the microspores visible; ×1620.

FIG. 30.—Vertical section of young microspore; nucleus has passed through a very short resting stage and has entered upon a prolonged prophase; ×1620.

FIG. 31.—Cross-section of young pollen grain; large central vacuole present and tube and generative nuclei at one side of pollen grain; ×1620.

FIG. 32.—Vertical section of slightly older pollen grain than fig. 31; generative cell lies near center of pollen grain; ×1620.

PLATE XV

FIG. 33.—Section of one of large multinucleate tapetal cells which occur within microsporangium after microspores have separated from tetrads; nuclei in various stages of disintegration; ×810.

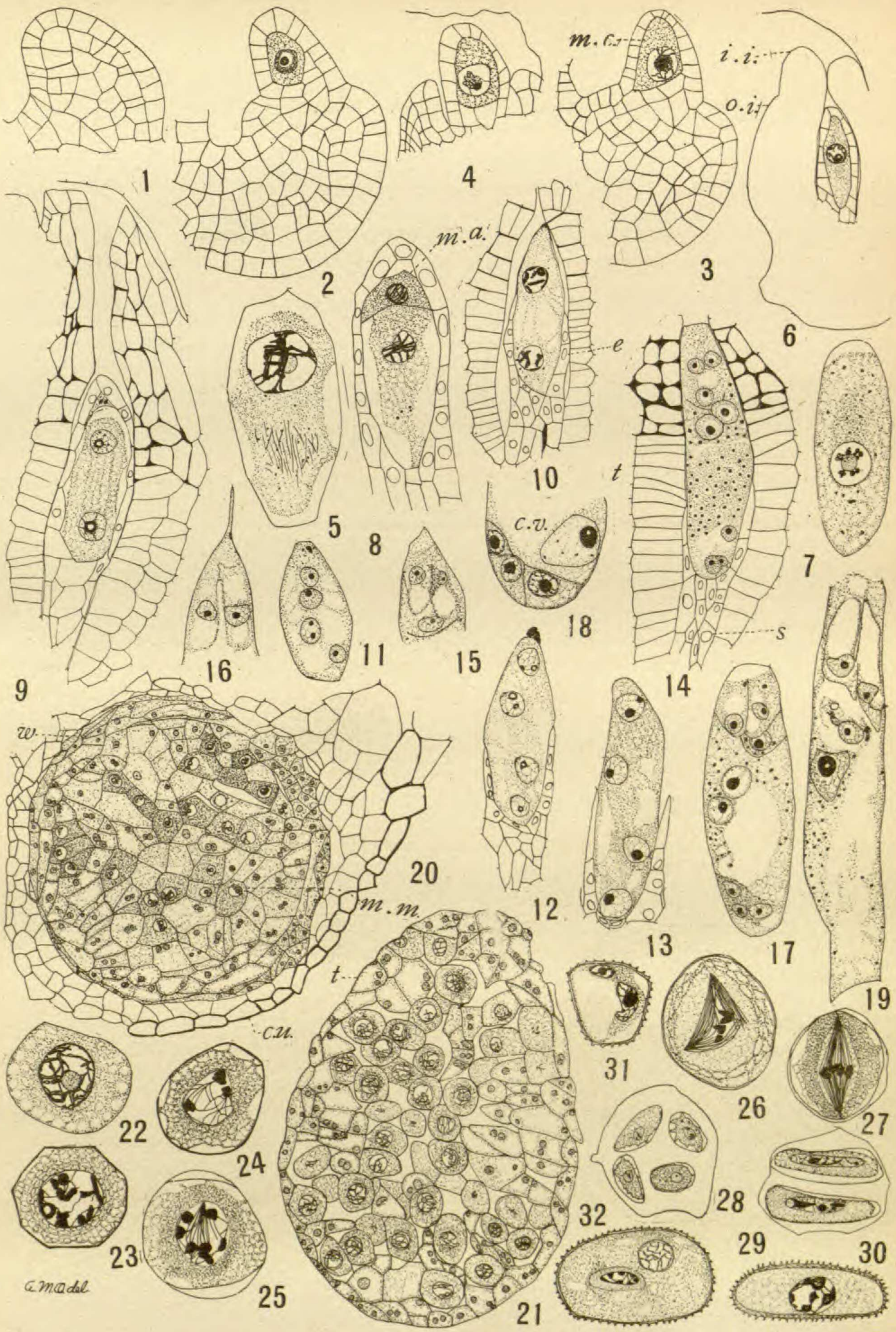
FIGS. 34, 35.—Vertical sections of nearly mature pollen grains; note change in size and cytoplasmic contents from fig. 32; cytoplasm packed full with food which is doubtless starch grains; in fig. 34 generative cell lies against wall of pollen grain, while in fig. 35 it lies free within cytoplasm of tube cell; ×1620.

FIG. 36.—Cross-section of mature pollen grain; cytoplasm of generative cell cannot be distinguished from that of tube cell; generative nucleus forms a crescent and contains 7 distinct chromatic masses; ×1375.

FIG. 37.—Vertical section of ovule after pollen tube has entered embryo sac; sketch is largely in outline and was derived from 3 serial sections; ×500.

FIG. 38.—Vertical section of micropylar region of embryo sac after pollen tube has entered sac; ×1000.

FIG. 39.—Two sperms near egg nucleus; ×1620.



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